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THE SPORULAR DEVELOPMENT OF THE AMEBÆ VILLOSA, LEIDY.

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In April, 1897, the writer secured some decayed leaves from a pond in the Audubon Park, in New Orleans, and on scraping a portion from one of the leaves, placing it under a cover-glass, and then examining it with a one-fourth objective, the field was seen to be filled with a number of *Amebæ villosa*, Leidy.

Some of the specimens were active, some were apparently on the threshold of encystment, while others had already entered that state.

The field, fortunately, was entirely free of other forms of amebæ, as well as of the troublesome *Paramecium*, Rotifera and worms.

For a while the field was thoroughly examined, and the writer noticing something unusual about the amebæ, concentrated his attention on one of the forms that had become quiet and was evidently about to become encysted. This specimen measured 1-175-inch, displayed the posterior well-covered with the villous processes which are diagnostic of this specie; the endoplasm contained a number of linear bodies and some food-balls already changed in color; the contractile vesicle was large and active, and instead of the usual nucleus, there were from ten to fifteen nuclear-looking bodies that moved freely in the endoplasm in unison with a slight contraction and expansion of the body. These nuclear-looking bodies were evenly dispersed, of a bluish tint, globular, very granular and in size varied from 1-2750

to 1-1800-inch. The slight contraction of the body became fainter, and in about one hour there was a rapid movement of the contents of this specimen, to the posterior extremity, and at the same time a rupture of the seemingly dense ectoplasm of this part; a number of the nuclear-looking bodies, in company with the linear bodies and food-balls, were ejected from the body with considerable force, sending them a distance from the body, equaling one-half of its long diameter. The ameba now seemed to collapse and the contractile vesicle disappeared.

The writer's attention was now confined to the nuclear-looking bodies that lay scattered about. In the course of a few minutes, the granules contained in these bodies became partially concentrated in one place in contact with the ectoplasm, and was of a deeper blue in color; this concentration of the granules left more than one-half of each body almost clear and transparent, and in this clear space there appeared simultaneously with the concentration, a very minute but distinct pulsating vesicle; in a short time a slight movement of the body was detected and there appeared a flagellum equaling in length from four to five of the body's diameters, and was directed stiffly forward; the body now became very active and in a few seconds darted off in a rapid chase about the field, in an aimless manner, reminding the writer of the zoospores of the *Achlya prolifera*. Casting a glance at the other free nuclear-looking bodies, it was seen that most of them were undergoing the same change, and they were kept under observation until they had all disappeared from the field, in the same manner. It was impossible to follow any one of these zoospores, as the field had become filled with them.

The writer now confined his attention to one of the encysted amebæ. The one selected measured 1-250-inch, possessed no trace of a contractile vesicle, no food balls, a few of the linear bodies, some of the nuclear-looking bodies and nothing that could be differentiated as the original

nucleus ; the nuclear-looking bodies were granular, as the ones cited above, and instead of being free in the endoplasm, were congregated in five spherical masses, each mass being composed of from four to six units and was enclosed in a very distinct membrane, which was made even more distinct by adherent granules.

In a short time and without any apparent movement of the body, three of these spherical masses were thrown out with some force, the fissure in the ectoplasm of the encysted ameba was not closed, and the whole form collapsed, still containing two of the masses.

In about fifteen minutes after being ejected, the membranous coverings of the units were ruptured and the contained nuclear-looking bodies were freed.

The average size and appearance of these bodies were the same as the ones seen discharged from the ameba first recorded.

In the course of a few minutes they were seen to go through identically the same phenomena as was observed to take place with the one first mentioned.

The field was now filled with these zoospores, and being free from all other forms of life, offered a good opportunity for further study.

In about three hours after beginning the observation some of the zoospores had slackened their movements, would come to a halt for a short time, and then start off again ; a number were less active than the rest and very soon became quiescent. Selecting a quiet specimen that measured 1-2000-inch, and using a one-eighth objective it could be distinctly seen to elongate itself and then resume its original size ; would throw out a single minute lobate process now from one side and again from the other side. The dark blue mass of aggregated granules, first observed in the nuclear-looking bodies after they had been ejected from the ameba, had become much smaller and now represented the nucleus, the contractile vesicle was very distinct and the intervals between

diastole and systole were short. This extrusion of lobate processes was witnessed for some time, and it was noticed that there was no change in the position of the young ameba, but that after awhile it retained the elongate form and would throw out pseudopodia from all parts of its body that would at times exceed the length of the zooid ; at these times it had the appearance of a minute *Ameba proteus*. Many of the forms measured now as much as 1-900-inch, without the pseudopodia. The hour being late, the slide was carefully prepared and put away with the view of continuing the observation on the following evening.

On resuming the observation, nineteen hours afterwards, the field was found strewn with a very large number of small and active amebæ that differed from the larger forms of *Amebæ villosa*, only in the absence of the villous processes ; the endoplasm was slightly granular, the nuclei and contractile vesicles as distinct as in the large forms ; they were freely moving about and extruded only the lobate processes. Measurements showed them to range from 1-800 to 1-550-inch.

In two places on the slide were a number of forms, from ten to fifteen, closely huddled together, as if dropped in a mass at that place. In size and shape they were the same as the free moving ones ; the nuclei, contractile vesicles and anterior clear spaces were exceedingly distinct ; they had a slight movement on and alongside of each other, without seeming to increase the space occupied by them. They reminded the writer of a litter of kittens a day or two old. The writer in speculating on this phenomenon, came to the conclusion that those nuclear-looking bodies that remained in the ameba after a part had been ejected, were developed within the confines of the body, and were freed only after the dissolution of the firm ectoplasm, and in this way the clusters of amebæ were produced.

The slide was now again laid aside, and on resuming the observations, eighteen hours afterwards, very few forms

were found, and they differed in no way from the forms seen the evening before. The writer believes that if food could have been supplied, the observation could have been extended so as to witness the full development of these young forms.

The literature on the development of the ameba, in the possession of the writer, is very limited, and from it he gleans that up to 1891 the sporular development of these lowly forms was only glimpsed, and was not worked out as fully as has been done in this case.

To make this history of the sporular development of the *Amebæ villosa* (and by inference all amebæ) complete, there is only one essential requisite, and that is to trace the origin of the nuclear-looking bodies to the nucleus.